Electronic Supplementary Information

Taking a Hard Line with Biotemplating: Cobalt-Doped Magnetite Magnetic Nanoparticle Arrays

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Supplementary Methods for Protein Production

The Protein Sequence

The amino acid sequence of the mature form of Mms6 (Mms6 sequence available at Uniprot with identifier Q2W8R5) protein produced for these experiments:

MGSHHHHHHHHGSTENLYFQGCPRMGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSR DIESAQSDEEVELRDALA

The *N* terminus of the protein features the additional sequence MGSHHHHHHHHGSTENLYFQGCPRM which comprises an octa-histidine tag for purification, followed by a TEV cleavage site and single cysteine residue for Au attachment.

Producing the Expression Construct

The gene sequence encoding the mature form of Mms6 was amplified, using PCR, from *Magnetospirillum magneticum* AMB-1 genomic DNA using the primers 5'-CTAGGTTAGGCCAGCGCGTCGCGCAG-3' and 5'-CCTGCAGGATGCCAGCTCAGATCGCCAACGGAG-3'. The cleaned PCR product was digested with *Avr*II and *Sbf*I restriction enzymes (New England Biolabs) and ligated into a similarly cut plasmid pBPTNHT which encodes the *N*-terminal tag. The correct sequence was confirmed by gene sequencing.

Introducing the Cysteine Mutation

To introduce the cysteine residue, the following mutagenic primer pair was used:

mms6cysF 5'-CCTGTATTTTCAGGGTTGCCCTAGGATGGGTGGAAC-3'

mms6cysR 5'-GTTCCACCCATCCTAGGGCAACCCTGAAAATACAGG-3'

Using Kod hotstart DNA polymerase (Merck) and the primers above, the pBPTNHTmms6 plasmid was copied using the following thermal-cycling parameters:

Cycle step	Temp (°C)	Duration (sec)	Number cycles
Initial denaturation	95	120	1
Denaturation	95	20	↑
Primer annealing	55	20	16 repeats
Elongation	68	100	↓
Final Elongation	68	240	1

The product was digested with *Dpn*I restriction enzyme (New England Biolabs) and cleaned with a PCR cleanup kit before being introduced into XL10-Gold (Agilent) *E. coli* cells. A single colony was isolated and the plasmid sequenced to ensure the insertion of the desired cysteine codon.

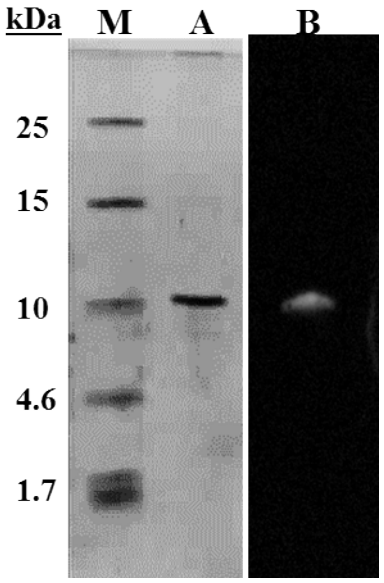

Figure 1S - Coomassie stained SDS-PAGE and corresponding western blot of purified His8-cys-Mms6. Lane M is the molecular weight marker with approximate masses shown in kDa. Lane A is the stained protein gel and lane B the western blot.

POFHK Reaction Performed on a Clean Gold Surface and a PEG Coated Gold Surface

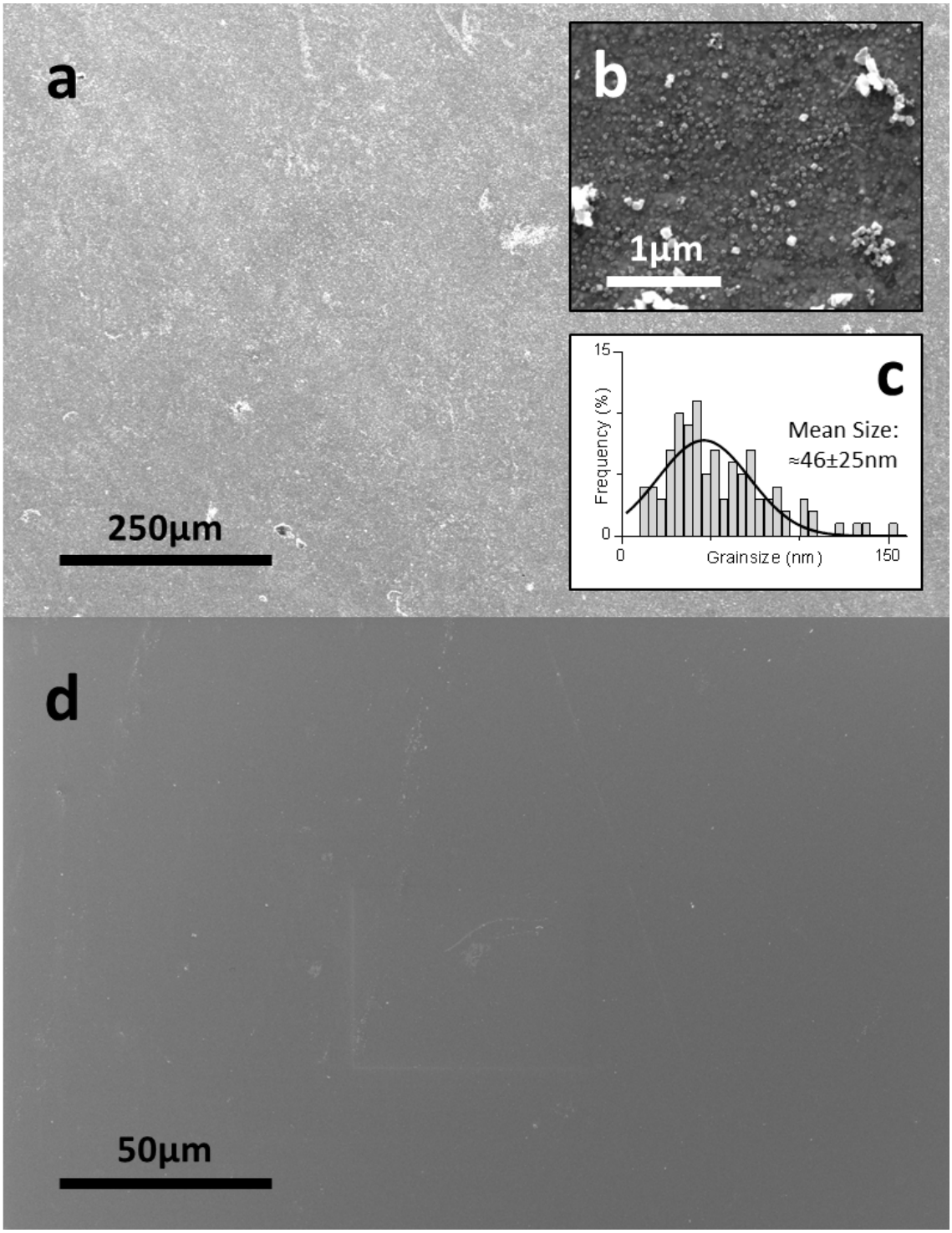

Figure 2S – SEM images of a clean gold surface (which underwent no surface patterning) (a and b), and of a gold surface immersed in a 1M PEG in ethanol solution for >16 hours (d) that were subject to a POFHK reaction designed to form magnetite. A large number of particles were found to form on the clean gold surface, with only a small number forming on the PEG coated gold surface. A low density layer of small nanoparticles (<40nm) formed on the clean gold surface with some collections of larger particles, and grain size analysis (c) shows that the nanoparticles which formed had a much larger size distribution (≈25nm) and a smaller mean size (≈46nm) than the particles which formed on the cys-Mms6 patterned surfaces.

Magnetic Force Microscopy (MFM) of 6% Cobalt-Doped MNP Arrays Biotemplated by Mms6 Immobilised onto Gold Surfaces via EDC/NHS Attachment (6%$Co_{surface}$EN)

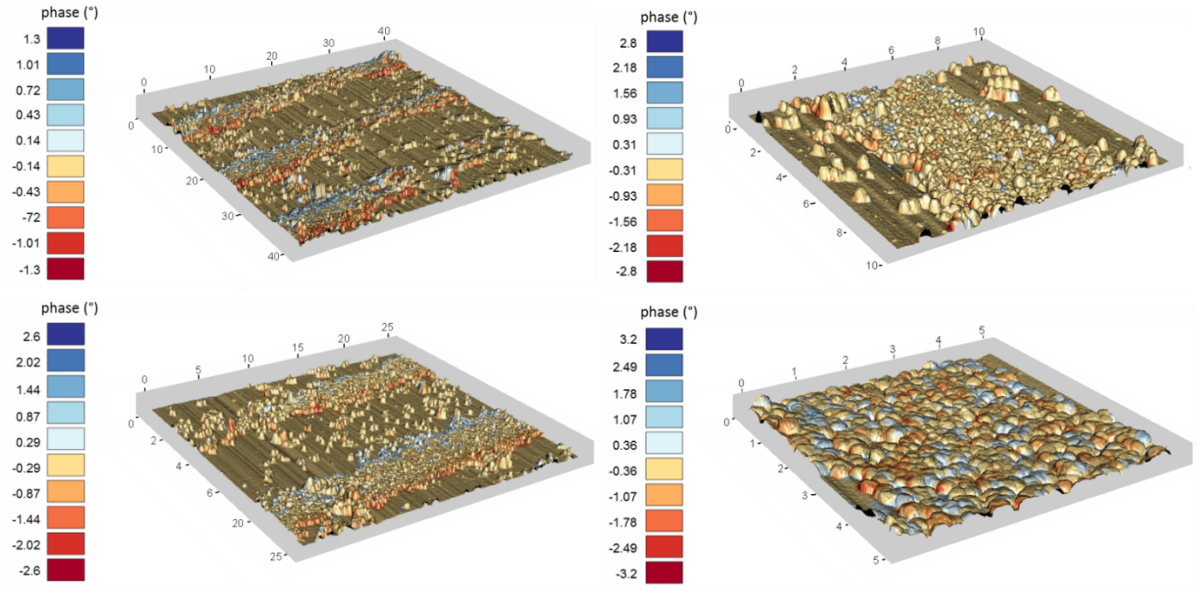

Figure 3S - Composite images of topography obtained with tapping mode AFM and MFM phase shift at a lift height of 50nm of 6% cobalt-doped magnetite MNPs biotemplated by Mms6 immobilised via EDC/NHS attachment[1] onto gold (x and y scales are in µm). These images of lines with a width of ≈5µm show that zones of attraction and repulsion (red and blue areas) can extend over large areas. This could be an effect of the 6% cobalt-doping, which increases the coercivity of the MNPs. Therefore, the direction of magnetisation is harder to perturb at room temperature, and the cobalt-doped MNPs may be able to form more stable interactions on the 2D gold surface than undoped magnetite MNPs.

[1] Experimental details can be found in the following reference: J. M. Galloway, *Biotemplating arrays of nanomagnets using the biomineralisation protein Mms6*, PhD thesis, University of Leeds, 2012.

Energy Dispersive X-ray (EDX) Analysis

EDX spectra and maps for gold, oxygen and iron were obtained with an Oxford Instruments AztecEnergy EDX detector system attached to Hitachi SU8230 SEM, with an accelerating voltage of 7 keV and working distance of approximately 15 mm. Images were processed with Inca software and samples were mounted onto aluminum subs using double sided carbon tape and earthed with silver paint.

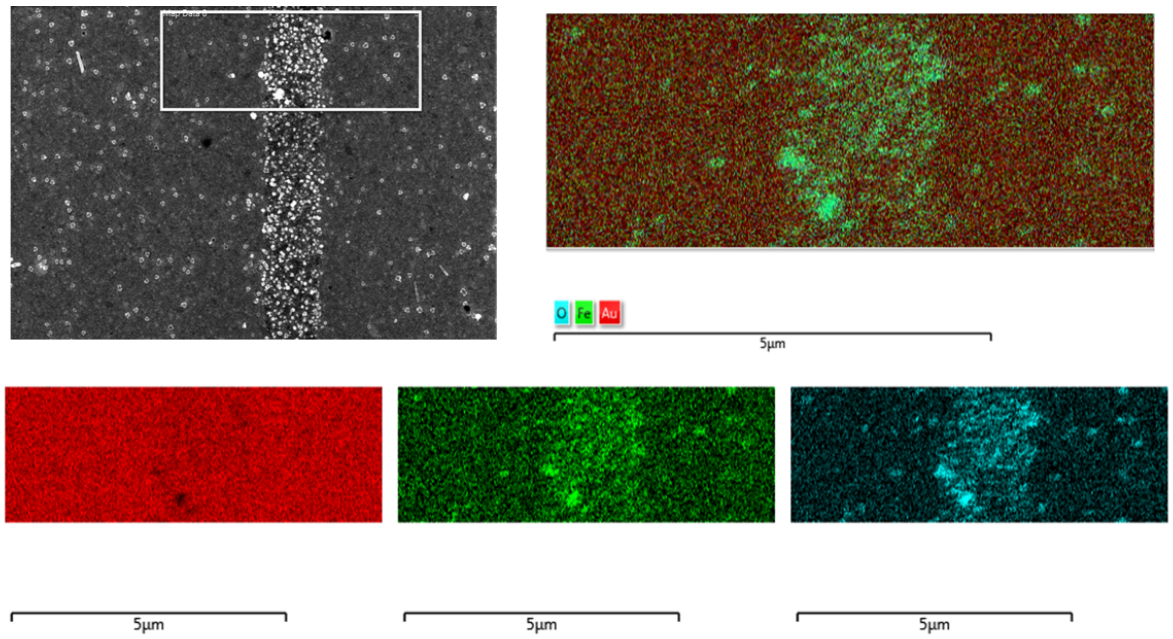

Figure 4S - SEM image and corresponding EDX maps of gold (red), iron (green), oxygen (blue) and an overlaid map (top left) of 6% cobalt-doped magnetite nanoparticles biotemplated by Mms6 onto gold. These images reveal that high levels of iron and oxygen were detected where a high density of nanoparticles formed. It was not possible to resolve a map for cobalt, as the peak for Co Lα (776 eV) significantly overlaps with that of Fe Lα (705 eV). As there is only a small amount of cobalt in the 6% cobalt-doped samples, the signal from the iron dominates the signal detected.

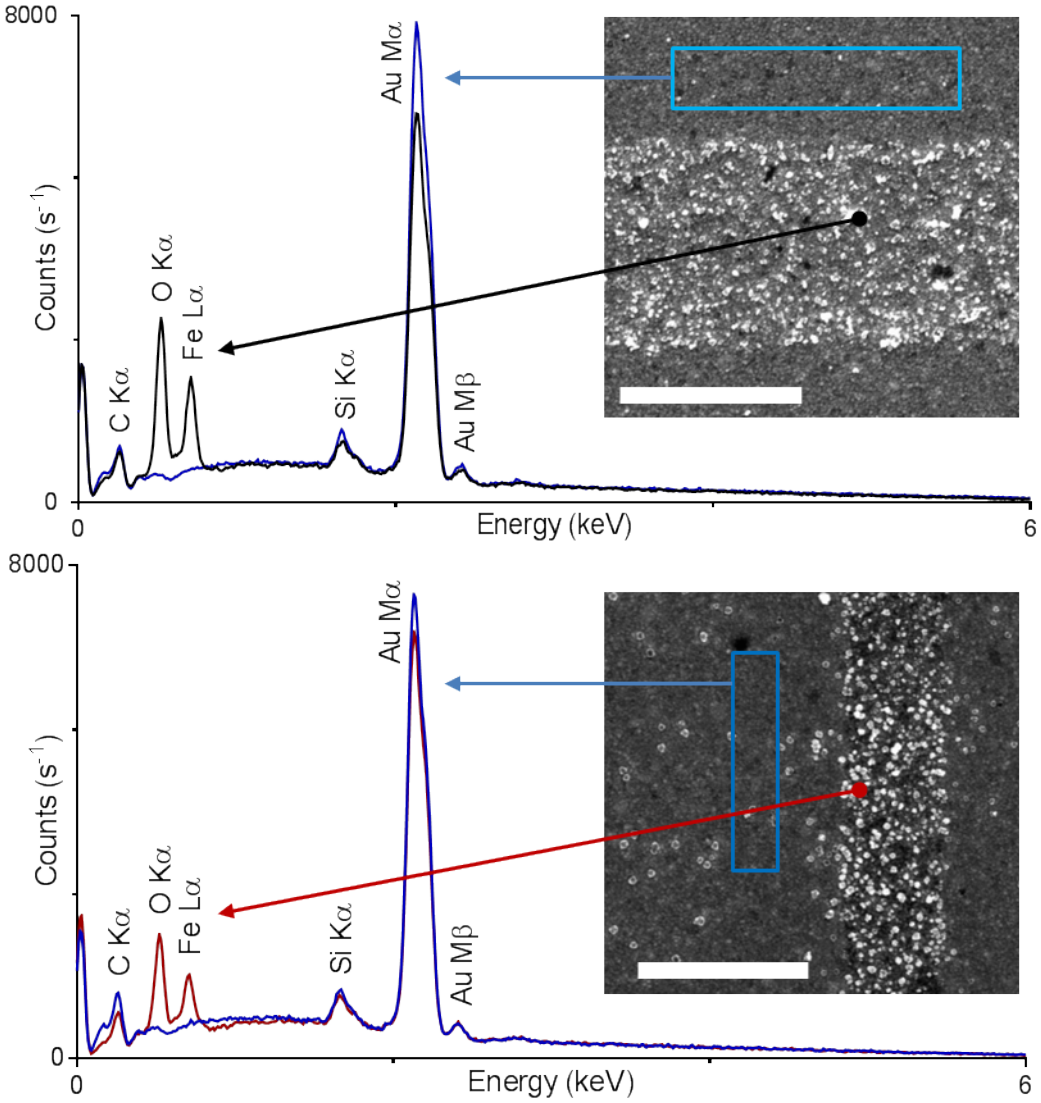

Figure 5S – EDX spectra and corresponding SEM images (scale bar top image 1 µm, bottom image 2 µm) of magnetite (top image and spectrum) and 6% cobalt-doped magnetite (bottom image and spectrum) nanoparticles biotemplated by Mms6 onto gold. X-rays collected over the wide anti-biofouling PEG SAM background area (blue boxes and spectra) reveal the presence of much less iron and oxygen than a spectrum taken from where a dense region of particles formed (shown in black for magnetite and red for 6% cobalt-doped magnetite). It was not possible to resolve a peak for cobalt, as the peak for Co Lα (776 eV) significantly overlaps with that of Fe Lα (705 eV). As there is only a small amount of cobalt in the doped samples (6%), the signal from the iron dominates the signal detected.

X-ray Diffraction (XRD)

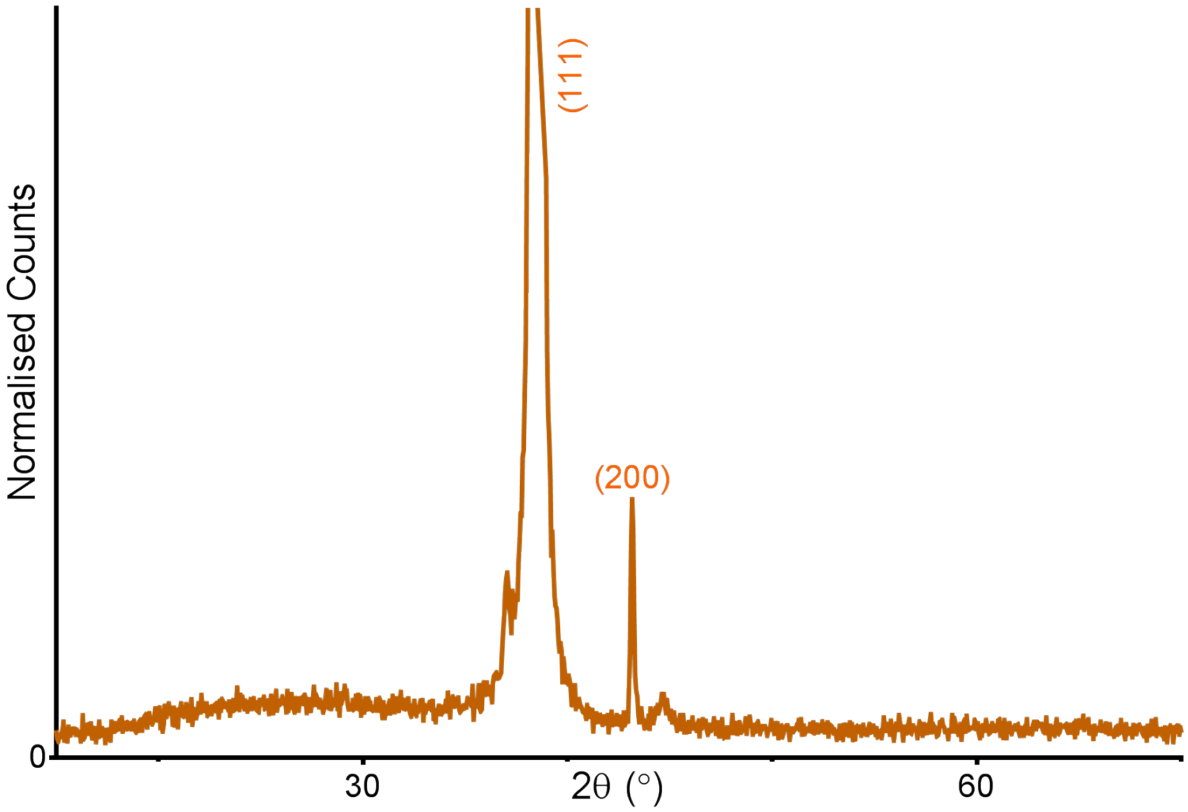

Figure 6S – XRD spectra of a gold substrate (without any PEG, cys-Mms6 or MNP mineralisation). The gold peaks are labelled.

X-ray adsorption analysis of MNP Arrays Biotemplated by Mms6 Immobilised onto Gold Surfaces via EDC/NHS Attachment ($Fe_{surface}EN$ & $6\%Co_{surface}EN$)

X-ray magnetic circular dichroism (XMCD) experiments were performed on the Beamline for Advanced Dichroism Experiments (BLADE) I10 at the Diamond Light Source, UK. $Fe_{surface}EN$ and $6\%Co_{surface}EN$ samples[2] were fixed to a copper sample holder with conductive paint and placed in the adsorption end station. All measurements were performed at room temperature in ultra-high vacuum conditions. As the biomineralised surface had incomplete coverage, a map of the Fe edge was made using the sample x-y stage. Further measurements were made at the area of strongest Fe signal. For XAS measurements, linearly polarised x-rays were used with an energy range 700-730 eV in steps of 0.1eV for the Fe $L_{2,3}$ edge and for 770-810 eV for the Co $L_{2,3}$ edge. Data shown here are the measured drain currents that run back into the sample on illumination with soft x-rays.

For XMCD studies, a magnetic field of ±0.3 T was applied and a fixed circularly polarised light was used. The XMCD signal was generated by subtracting the measurements made by reversing the applied magnetic field. Data aggregation and background subtraction was performed in R using a custom written processing code. Further fitting of the data was completed with CTM4XAS software.[3]

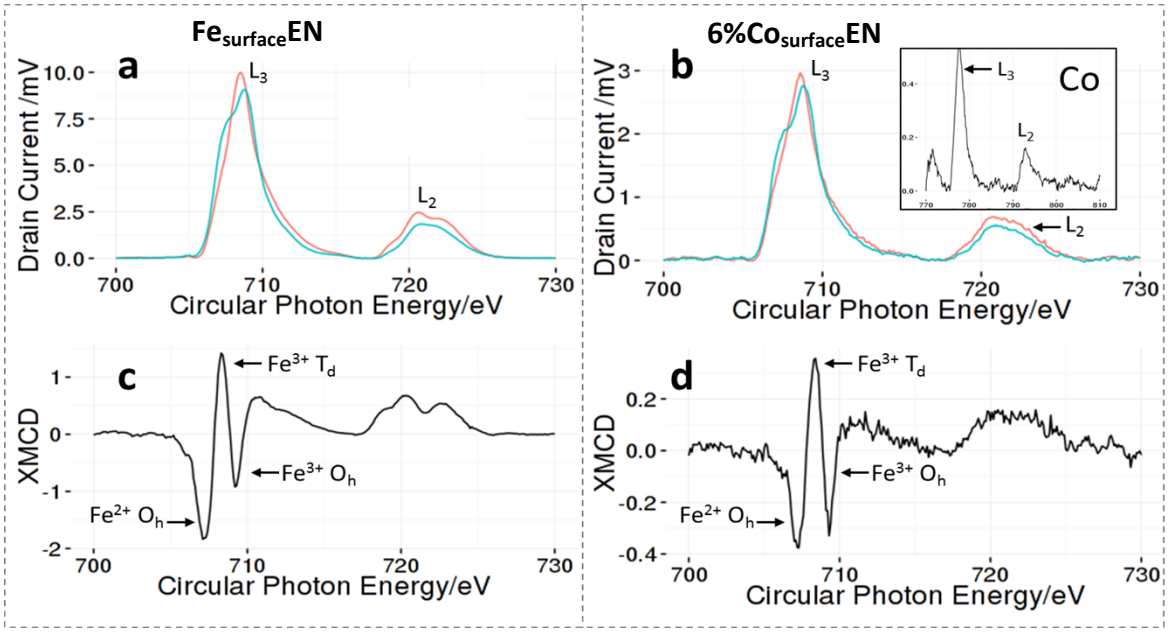

Figure 7S – X-ray absorption spectra (XAS) (a and b) near the Fe (a and b) and Co (inset of b only), and X-ray magnetic circular dichroism (XMCD) spectra (c and d) of MNP arrays of $Fe_{surface}EN$ (c) and $6\%Co_{surface}EN$ magnetite (d) (biotemplated by Mms6 immobilised by EDC/NHS attachment onto gold).[1] The XMCD spectra (c and d) is the difference between the X-ray adsorption spectra displayed here near the Fe L-edges recorded in magnetic fields of ±0.3 T (red and blue lines in a and b). The intensities of the three peaks at the L_3 adsorption edge of Fe can be related to the relative concentrations of the different Fe sites in magnetite (with the Fe^{2+} and Fe^{3+} octahedral (O_h) sites and the Fe^{3+} tetrahedral (T_d) labelled in c and d). The first peak in the iron spectrum of the 6% cobalt-doped sample is reduced when compared to the undoped spectrum, and it is believed that this is a result of the partial substitution of Co^{2+} ions for Fe^{2+} ions at the octahedral sites in magnetite.

[2] Experimental details can be found in the following reference: J. M. Galloway, *Biotemplating arrays of nanomagnets using the biomineralisation protein Mms6*, PhD thesis, University of Leeds, 2012.

[3] E. Stravitski & F. M. F. de Groot, *The CTM4XAS program for EELS and XAS spectral shape analysis of transition metal edges*, 2010, Micron, 41, 7, 687-694

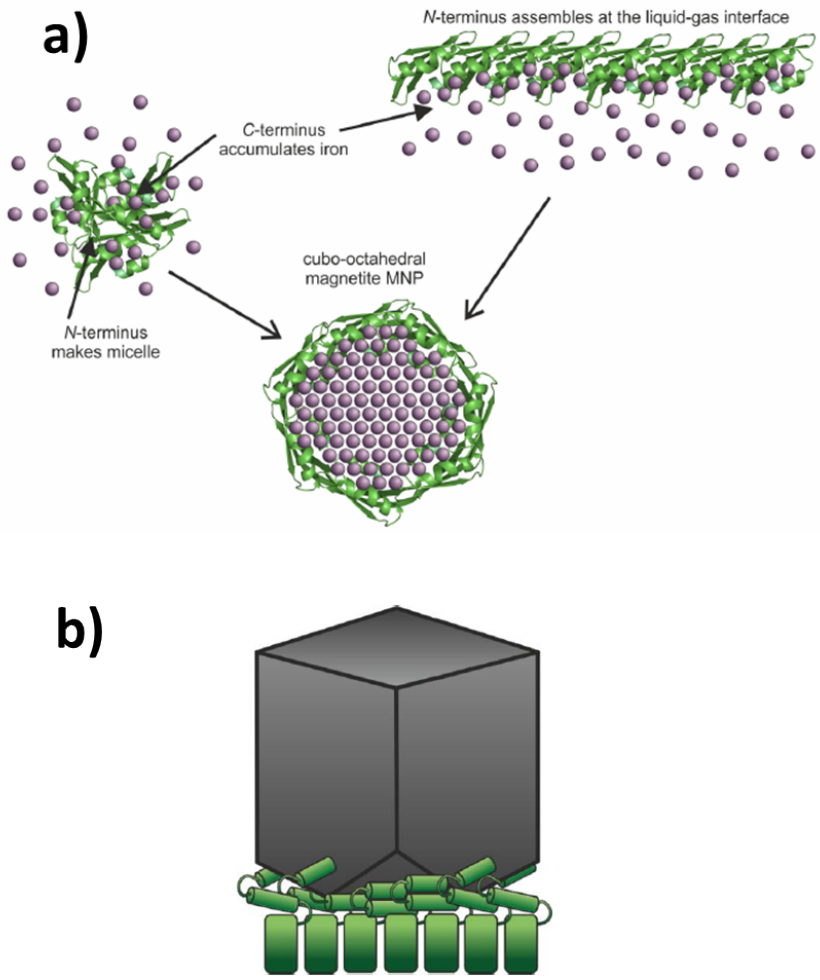

Figure 8S[4] - a) Illustration of the self-assembly of Mms6 in an aqueous solution (not to scale). Model of Mms6 generated using Quark[5] and cartoon rendered using PyMOL.[6] It is likely that Mms6 self assembles via the hydrophobic *N*-terminal region, either into micelles or at the liquid-gas interface. Therefore, the *C*-terminal section is able to interact with the mineralisation solution to form cubo-octahedral magnetite nanoparticles *in vitro*. b) An illustration of how Mms6 (green) could biotemplate magnetite cubes when immobilised onto a surface (not to scale). The hydrophobic *N*-terminus of Mms6 is represented by a rectangle and the hydrophilic *C*-terminus by two cylinders. When the protein is immobilised to a substrate via its *N*-terminus, the *C*-terminus is free to interact with the mineralisation solution and template magnetite. Mms6 is likely to only be able to template the underside of the MNP, so the particles appear cubic when imaged in SEM from above.

Vibrating Sample Magnetrometry (VSM)

[4] Further discussion can be found here, and images adapted from figure 7.1 and 7.2: J. M. Galloway*, Biotemplating arrays of nanomagnets using the biomineralisation protein Mms6*, PhD thesis, University of Leeds, 2012.

[5] D. Xu & Y. Zhang, Ab initio protein structure assembly using continuous structure fragments and optimized knowledge-based force field, *Proteins*, 2012, 80, 1715-1735.

[6] L. L. C. Schrödinger, The PyMOL Molecular Graphics System, Version 1.5.0.1, 2012.

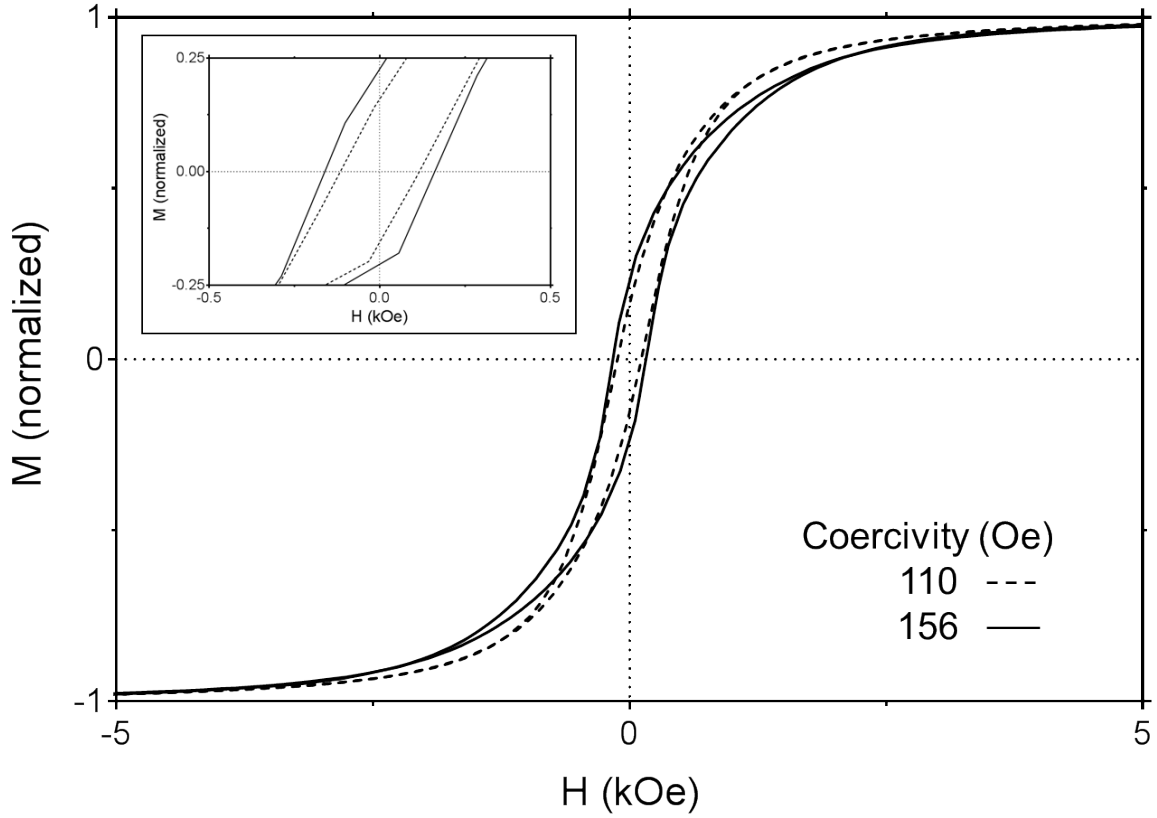

Figure 9S - Magnetic hysteresis loops recorded using VSM at 295 K of the $Fe_{Surface}$ MNPs (solid line) and Fe_{Bulk} MNPs (dashed line) . These loops show an increase in coercivity for the surface Mms6 biotemplated MNPs (156 Oe) when compared to the MNPs which formed in solution (110 Oe) during a POFHK reaction designed to form magnetite.

Magnetic Force Microscopy (MFM)

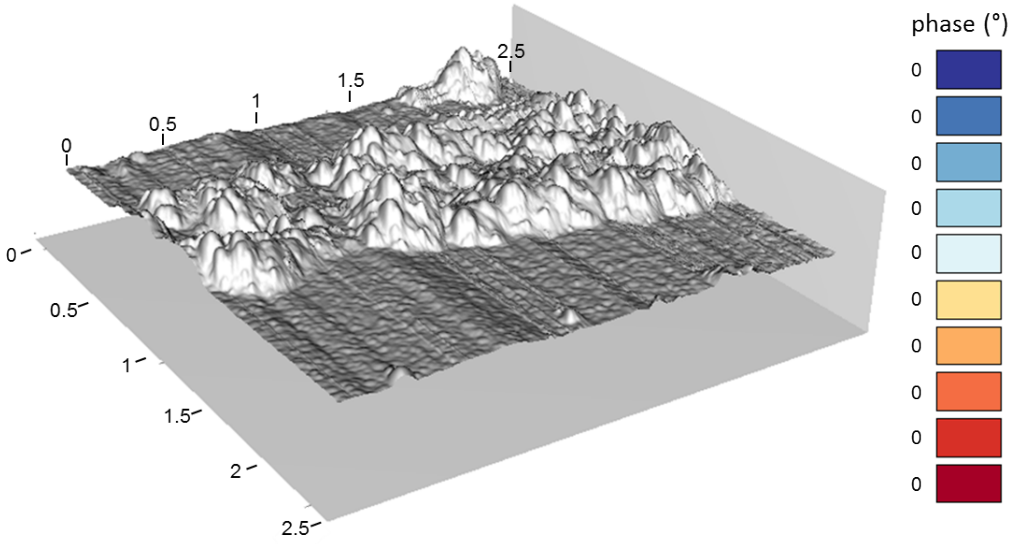

Figure 10S – Composite images of topography obtained with tapping mode AFM and MFM phase shift at a lift height of 50nm of magnetite MNPs biotemplated by Mms6 onto gold (x and y scales are in µm). In this case a non-magnetic magnetic TESPA-V2 tip (Bruker) was used, and as a result no phase shift was detected. Hence the image appears colourless.

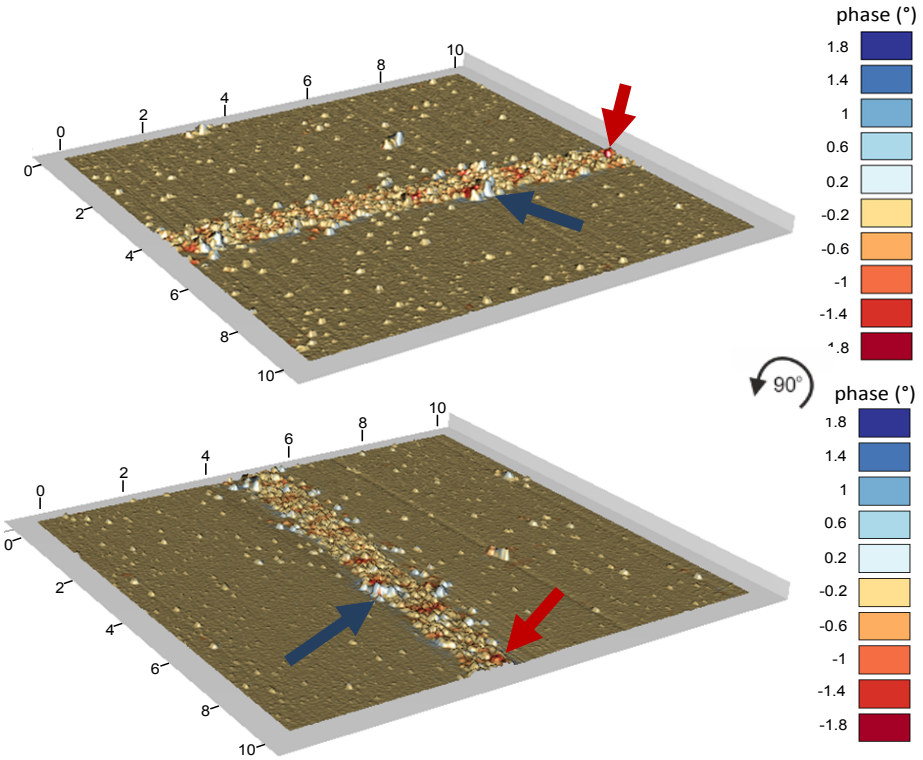

Figure 11S - Composite images of topography obtained with tapping mode AFM and MFM phase sift at a lift height of 50nm of magnetite MNPs biotemplated by Mms6 onto gold (x and y scales are in µm). These images were recorded on the same area of the sample, but the scan direction was rotated anticlockwise by 90˚. Both images show the same areas of attraction and repulsion (red and blue areas, with some example areas highlighted by red and blue arrows), suggesting that these biotemplated MNPs are ferrimagnetic and able to maintain their magnetic orientation at room temperature.